IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: CROOKS et al.

Title: ANALGESIC USES OF NORKETAMINE AND KETAMINE/NORKETAMINE PRODRUGS

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Declaration Under 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Mark S. Kleven, Ph.D., do hereby declare as follows.

2. My educational background, research experience, work experience, and professional memberships are described below.

3. My educational background is as follows:

Ph.D., Pharmacology, University of Minnesota School of Medicine, Minneapolis, MN, 1986. NIH Pre-Doctoral Fellow.
M.A., Physiological Psychology, Marquette University, Milwaukee, WI, 1979.
B.A./B.S., Psychology, Biology/Applied Mathematics, Bemidji State University, Bemidji, MN, 1977. Honors Scholar (1974-1977); B.A., summa cum laude.

4. I have the following research experience and have published more than 70 peer reviewed articles, most in high impact journals.

In vivo/ex vivo neurochemistry, radioimmunoassay, immunohistochemistry, and receptor binding. Stereotaxic surgery (rats); in vivo brain microdialysis (rats); chronic

indwelling cardiac catheters (rats and monkeys); ex vivo brain dissection (mice, rats and monkeys). In vivo, site-specific knock-down of gene expression using antisense and siRNA.

Operant behavior: conflict-procedure (pigeon); drug discrimination (mice, monkeys, pigeons, and rats); drug self-administration (monkeys, rats); conditioned avoidance responding (rats).

Unconditioned behavior: hypophagia (rats); stimulant-induced behaviors (mice, monkeys and rats); catalepsy (mice, monkeys and rats); 5-HT1A agonist-induced behaviors (rat); DOI-induced head twitches (rat); prepulse inhibition (rat); locomotor activity (mice, monkeys and rats), forced-swimming test (rat); tail suspension test (mouse); apomorphine-induced climbing (mouse); social-isolation (rat); dopamine-induced eye blinks (monkeys).

5. My work experience is a follows:

Yaupon Therapeutics, Inc. (2006-)

Vice President Preclinical Development

Direct GLP and non-GLP Efficacy, Safety, and Toxicology studies conducted primarily by CROs, but also by Yaupon staff, supporting IND submissions. Manage out-sourcing of IND-enabling Safety/Toxicology studies and have a key role for compiling new STTRs, IND and EMEA submissions. Am currently PI on an SBIR grant supporting a Phase I clinical trial for Attention Deficit Hyperactivity Disorder. Ongoing projects involve treatment of smoking cessation, methamphetamine abuse, ADHD, neuropathic pain, psoriasis, and cutaneous T-cell lymphoma.

Consultant (2005)

Behavioral Pharmacology / Preclinical development

University of Pennsylvania, Dept. of Psychology, Philadelphia, PA (2005-)

Visiting Scholar

Ongoing support and development of data gathering tools and analysis of in vivo electrophysiology and behavioral experiments. For example, assist in data analysis associating behavioral events with neuronal activity.

Scientific Commercialization LLC., (2004)

Principal, Head Early Phase R & D

Consulted and authored reports on treatment of coronary heart disease, e.g., aspirin resistance, antiplatelet activity of ADP inhibitors and PPAR-γ agonists. Assisted advice for a critical sNDA review and developed leads for a new line of business.

Pierre Fabre Médicament, CASTRES, France (1992-2004)

Institute de Recherche Pierre Fabre

Pierre Fabre Médicament, the pharmaceutical branch of Groupe Pierre Fabre, is France's second-largest private drug firm, allocating 140 million euros (ca. 23% of revenues) to research and development.

Head, Behavioral Pharmacology, Neurobiology II (1992-2004)

Chargé de Mission, Fonction Support (2002-2004)

Directed pivotal in vivo studies leading to clinical development of more than six new chemical entities, (e.g., F15063 and others, e.g. eptapirone that are in Phase I-II clinical trials). Developed and implemented novel in vivo behavioral screening assays for schizophrenia, depression, and anxiety. Implemented in vivo gene-silencing constructs and intra-cranial delivery methods (e.g., siRNA and antisense).

The University of Chicago, Dept. of Pharmacological and Physiological Sciences,

Pritzker School of Medicine, Chicago, IL (1986-1992)

Assistant Professor (Research Associate (1989-1992)

Research Associate (1987-1989)

NIMH Post-Doctoral Fellow, Psychiatry (1986-1987)

Co-Principal Investigator on studies of pharmacological effects of acute and chronic cocaine in primates. Assisted direction of studies demonstrating the ability of dopamine D1 antagonists to block cocaine self-administration and its subjective effects in primates. Developed efficient behavioral methods in primates to test compounds for treatment of cocaine abuse. Principal Investigator on studies of cocaine neurotoxicity in rats. Characterized neurotoxic properties and structure-related activity of amphetamine-related compounds, such as the anorectic fenfluramine.

6. I am a member of the following professional organizations:

American Chemical Society,
American Society for Pharmacology and Experimental Therapeutics;
Collegium Internationale NeuroPsychopharmacologicum (Fellow);
College on Problems of Drug Dependence (Industrial Relations Committee: 1997-2000);
Drug Information Association;
European College of Neuropsychopharmacology;
European Behavioral Pharmacology Society (Executive Committee: 2001-2005);
Society for Neuroscience

7. I am providing this Declaration to show the unexpected reduction of side effects occurring with administration of S(+) norketamine or R(-) norketamine in comparison to equipotent amounts of R,S(±) ketamine.

Drugs and Animals

8. For the below experiments, S(+)-norketamine hydrochloride, R(-)-norketamine hydrochoride and (±) ketamine hydrochloride were dissolved in saline and administered by an intraperitoneal (IP) (1ml/kg) route. Doses refer to salt forms. Male (about 350 g) Sprague-Dawley rats were used.

Chronic Constriction Nerve Injury (CCI) model and Equipotency

9. In appended Figure 1 a rat chronic construction nerve injury model shows equipotent dosage amounts to be as follows:

16 mg/kg for S(+)-norketamine,

32 mg/kg for R(-)-norketamine and

8 mg/kg for (±)-ketamine.

Dose-related antihyperalgesic effects of S(+)-norketamine (S-NKET) occur after the intraperitoneal route of administration (IP). Antihyperalgesia was assessed in a nerve-injured paw (i.e., following chronic constriction nerve injury, CCI) by a decrease in vocalization threshold (VT measured in grams of pressure applied to the paw) using a Paw Pressure Simulator Analgesia Meter. See Randal L., Selitto J. A method for measurement of analgesic activity on inflamed tissue, Arch Int Pharmacodyn Ther 1957; 111: 409-419. Percent maximum possible effect (at peak time), %MPE = (VT – predrug baseline / preCCI–predrug baseline) * 100; preCCI ≈ 200 g Mean ± SEM (n = 8 rats). The above equipotent amounts achieved a maximum positive effect against mechanical hyperalgesia, and therefore having an equivalent therapeutic effect, would be expected to produce equivalent side effects.

Side Effects at Equipotent Dosages

10. Ataxia (Figure 2A), stereotypic behavior (Figure 2B), and activity level (Figure 2C) following intraperitoneal administration (IP) of S-norketamine (S-NKET, 16 mg/kg), R-norketamine (R-NKET, 32 mg/kg), (±)-ketamine (KET, 8 mg/kg) at approximately maximum antihyperalgesic doses (see Figure 1). Data are presented as total scores (Behavioral Rating Scale; see Table 1). Saline served as control. Mean ± SEM (n = 4 rats, S- and R-NKET; n = 8 rats, KET).

11. The behavioral rating scale (Table 1, appended hereto) was used for quantification of ataxia, stereotypic behaviors and activity levels. Both S(+)- and R(-)-norketamine (IP) produced significantly less ataxia and stereotypic behaviors compared to (±)-ketamine at doses maximally effective against mechanical hyperalgesia (16, 32, and 8 mg/kg, respectively, in Figures 2A and 2B). Specifically, S(+)-norketamine produced 5.0 fold less ataxia, although it would have been expected to be equal to ketamine. R(-)-norketamine produced seven fold less ataxia, although it would have been expected to be equal to ketamine. Activity levels were less depressed for S(+)- and R(-)-norketamine in comparison to (±)-ketamine (Figure 2C). Of note, (±)-ketamine was observed to evoke an early (within 5 min) PCP-like behavior (e.g., head weaving, turning). This was not observed with S(+)- and R(-)-norketamine at any dose tested.

Methods of CCI model

12. For the chronic construction nerve injury model, the unilateral peripheral mononeuropathy was produced on the left hind limb according to the method described by Bennett G., Xie Y, A peripheral mononeuropathy in rat that produced disorders of pain sensation like those seen in man. Pain 1988: 33:87-107. Under pentobarbital anesthesia (40 mg/kg, IP) ligation to the sciatic nerve and sham surgery were performed in each rat on the left and right hind paws, respectively. Proximal to the sciatic trifurcation, the nerve (about 7 mm) was freed from adhering tissue and four loose ligatures were tied around the nerve (1 mm apart) using 4.0 chromic catgut, barely constricting the diameter of the nerve. In sham surgery, the right sciatic nerve was exposed using the same procedure, but the nerve was not ligated. The incision was closed in layers with silk thread 3.0. Rats showed a mild aversion of the affected paw and a mild degree of foot drop. No severe motor impairment was observed. Chronic constriction injury of the sciatic nerve (CCI) resulted in development of mechanical hyperalgesia (enhanced sensitivity to mechanical stimuli). This was evident by a decrease in vocalization threshold (VT, g; paw pressure test) compared to pre-surgical. Percent maximum possible effect (at peak time), %MPE = (VT – predrug baseline / preCCI–predrug baseline) * 100; preCCI ≈ 200 g Mean ± SEM (n = 8 rats).

CCI model data and statistics

13. Data for the CCI test were normalized for baseline values. Areas under the curves (AUC_{0-t}) were calculated for the normalized data by the trapezoidal rule. Dose-response curves were generated (%MPE as a function of log dose). The effective doses for a 50% maximum possible effect (ED_{50}) and 95% confidentiality limits (95%CL) were calculated using the method of Tallarida R.J, Murray R.B. Manual of pharmacological calculations with computer program, New York, Springler-Verlag, 1987; 26-31.

Data are the mean ± SEM for (8) rats.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated: 7/15/08

Mark S. Kleven, PhD

Table 1. Behavioral Rating Scale (Sturgeon et al., 1979, with minor modification)	
Rating	Description of Behavior
Activity level	
0	Activity no different from control
-1	Moderate Activity (25% stationary; walking with intermittent passes)
-2	Low activity (75% stationary)
-3	No activity (100% of the time stationary)
Stereotypic behaviors	
0	Repetitive movement no different from control
1	Head bobbing, sniffing
2	Swinging head side to side, mouth chattering
3	Shaking, twitching, weaving
Ataxia	
0	Coordinated movement, no different from control
1	Loss of balance when rearing, jerky movement
2	Cannot move beyond a restricted area, frequent falling to the side with attempted walking
3	Unable to walk

Figure 1

Dose-Response Curve for Norketamine Enantiomers and Ketamine in Chronic Constriction Nerve Injury Model Following Intraperitoneal (IP) Administration

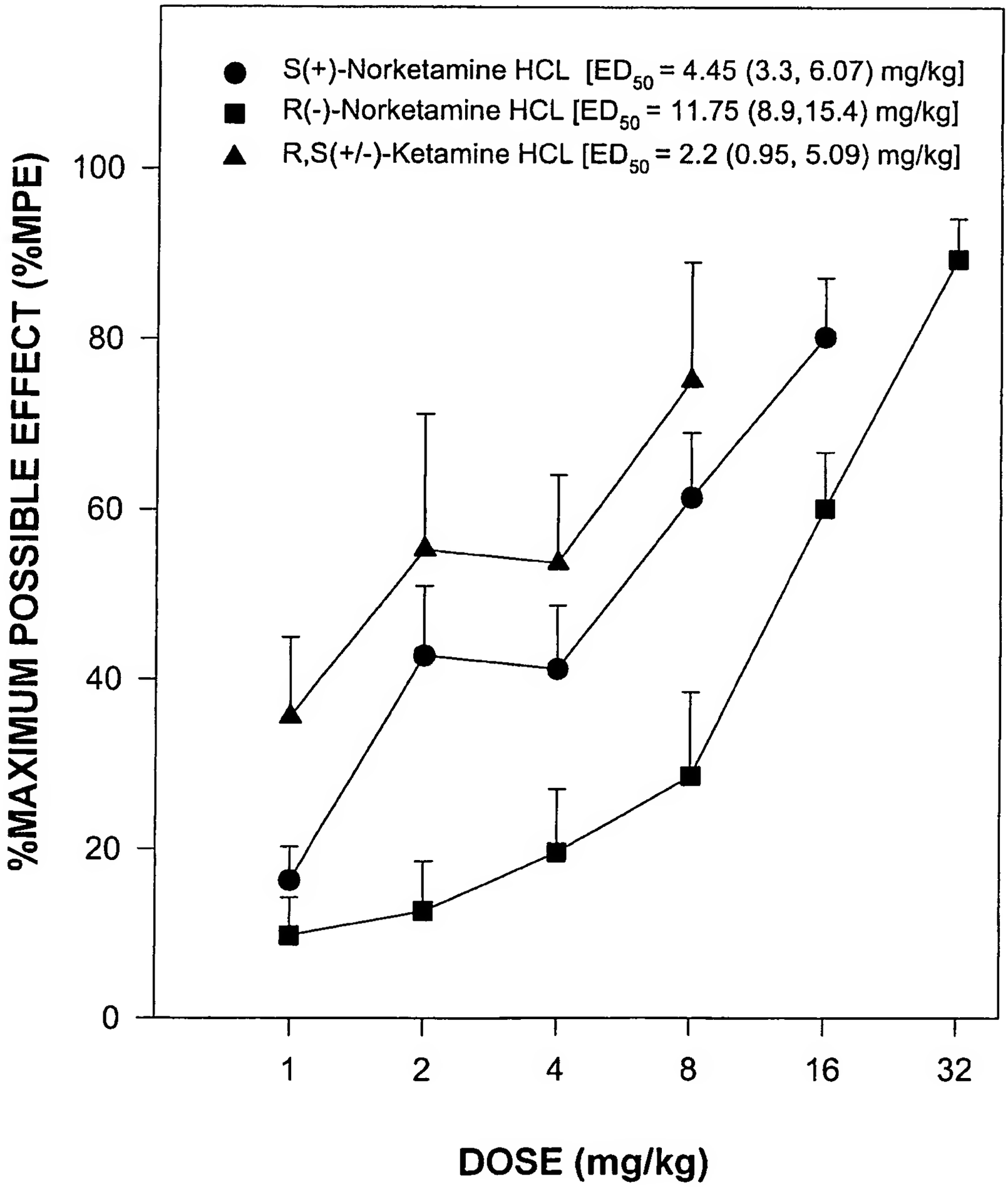
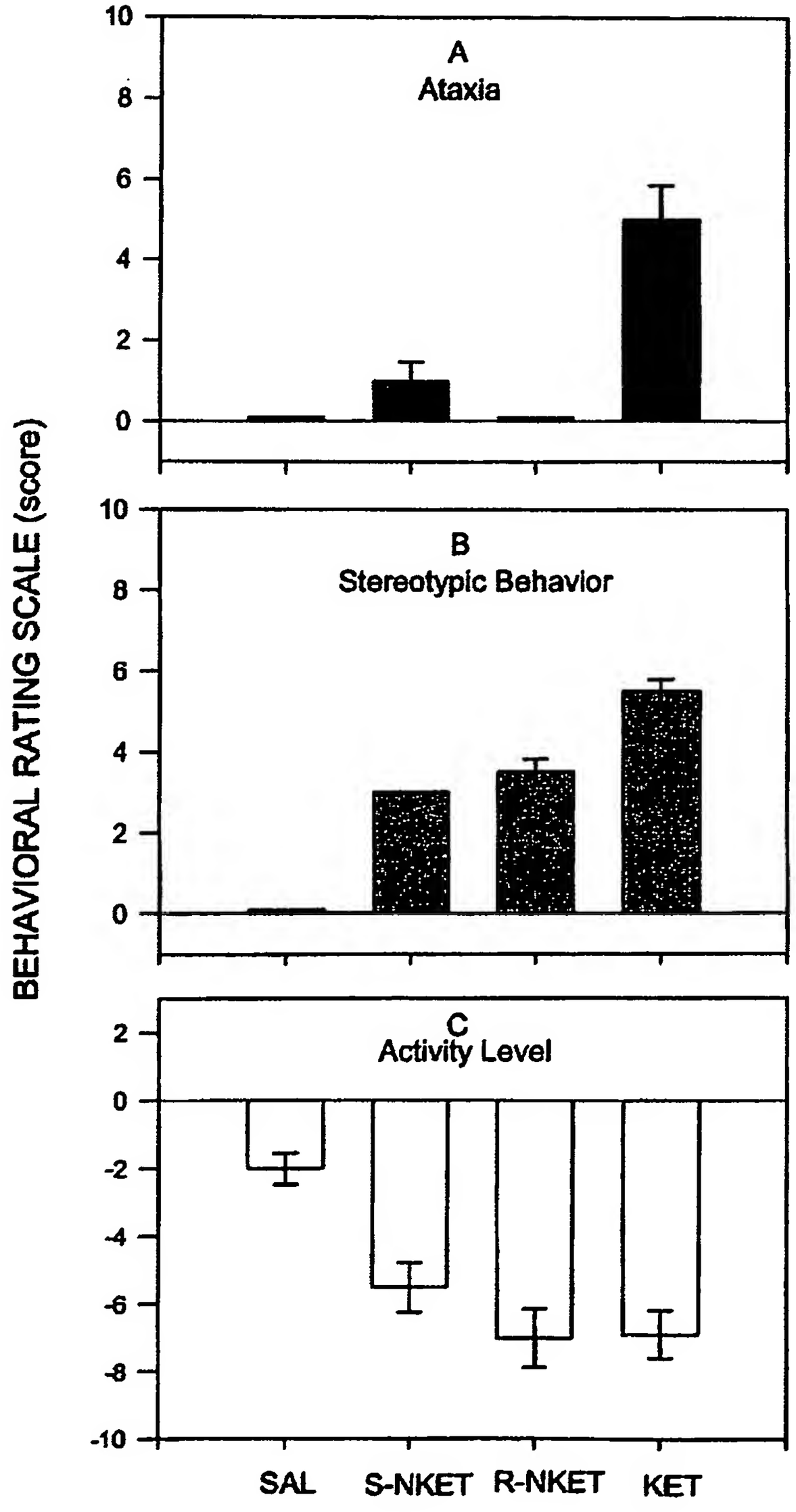


Figure 2


A
Ataxia
B
Stereotypic Behavior
C
Activity Level
BEHAVIORAL RATING SCALE (score)
10
8
6
4
2
0
-2
-4
-6
-8
-10
SAL
S-NKET
R-NKET
KET